International Journal of Agricultural
Science and Research (IJASR)
ISSN(P): 2250-0057; ISSN(E): 2321-0087
Vol. 5, Issue 1, Feb 2015, 29-42
TJPRC Pvt. Ltd.

EFFECT OF SALT STRESS ON PHYSIOLOGICAL ATTRIBUTES OF PEA (PISUM SATIVUM)

MANI. F

Laboratory of Horticol Sciences and in vitro Science, High Agronomic Institute Chott Mariem, Tunisia

Laboratory of Horticol Sciences, National Agronomique Institute of Tunisia, Tunisia

ABSTRACT

Ten pea (*Pisum sativum*) genotypes (Asgrow, Jumbo, Lincoln, Merveille de Kelvedon, Purser, Rajao Torpe, Rondo, Snajor Kosep Korai, Wando, and a Local variety) were used to study the effects of salt stress on the growth, photosynthesis rate, stomatal conductance, transpiration rate and chlorophyll contents. Pea seeds of different genotypes were grown in pots having fine sand as growth medium. After 30 days of germination, the plants were subjected to salt stress under 0, 25, 50 and 75 mM NaCl. At the end of the experiment, the plant growth was significantly decreased with increasing salinity. After one week of salt application, photosynthesis rate, transpiration rate, and chlorophyll contents of the plant were remarkably decreased with increasing salinity in all the genotypes. However, the Na ions accumulation was increased with increasing salt stress, which changed the Na:K ratio, and it seems to affect the bioenergetic processes of photosynthesis. Among different cultivars, the local variety, Lincoln and Merveille de Kelvedon were found to be salt tolerant whereas both Purser and Rajai Torpe showed salinity sensitive behaviour. Tolerant genotypes were successful in maintaining high plant dry matter, less concentrations of leaf Na, while non tolerant genotypes exibits high concentration of leaf potassium contents under the saline environment.

KEYWORDS: Pea, Photosynthesis, Transpiration, Chlorophyll Content, Na^+, K^+

INTRODUCTION

Pea (*Pisum sativum*) is an important edible leguminous seed crop for human nutrition. Its seeds contain 18-20% dry matter whose 10-12% is carbohydrate and 5-8% is protein (Vural *et a,* 2000). Pea is a cool-season vegetable crop of mild climate regions. Therefore, it gives higher yield in coldhumid regions compared to warm-dry areas. Its minimum temperature range for germination is between 1-6°C and it can survive in low temperatures up to - 5°C. Even though pea can grow in many soils, the best yield can be obtained in clay-loam, deep, productive, moist, slightly acid (pH 6.5-7.0) soils. When the soil is productive and moist, vegetative growth is advanced, on the other hand pea seed yield decreases. In addition, owing to its taproots, pea can use plants nutrients and water from different soil layers and increase organic matter content of soil.

As a legume crop, pea is able to fixate 50-150 kg ha^{-1} nitrogen from air (Ozdemir, 2006). Salinity is an abiotic stress that affects in pea the leaf growth, photosynthesis, mineral nutrition, stomatal conductance, transpiration, water and ion transport and increases sugars, amino acids and different ions along with acute effects on yield and quality. Salinity induced the disorders such as nonspecific chlorosis, stunted leaf size and impaired shoot growth (Levy and Syvertsen, 2004). Therefore, plants under saline conditions, adopt different mechanisms to adjust the osmotic and ionic

shocks caused by high salt stress.

Plants try to overcome the salinity induced nutritional and osmotic shocks by accumulating various organic (polyamines, proline, glycinebetaine etc.) solutes in their tissues (Ramanjulu and Sudhakar, 2001). However, among these osmoprotectents, proline and glycinebetaine (GB) compounds were produced in higher plants due to salinity (Yang *et al,* 2003) so, it is also considered as an adaptation to saline regimes. Since water on the earth contains the considerable ratios (30 g NaCl L^{-1}) of salts so it can be called as the saline planet and this NaCl affects the land structure as well as development of crops (Flowers, 2004). Both sodium and chloride produce many physiological disorders but chloride is the most dangerous because NaCl liberates almost 60% more ions into the soil solution than Na_2SO_4 (Cedra *et al,* 1982 ; Rachmilevitch *et al,* 2004). Excess of these salts also enhance the osmotic potential of the soil matrix and resultantly water intake by plants is restricted (Garcia-Sanchez *et al,* 2002).

As pea is not a halophyte so the above mechanisms are not well established. However, there are many other adaptations such as the absence of apoplastic pathways in roots, the elevated root/ shoot ratio and enhanced growth rate. Proline, glycinebetaine, salicylic acid, brassinosteroids, silicates etc. These chemicals play a vital role in osmotic adjustment under saline conditions, thus protects the plants under stressed conditions. So it's clear that pea is sensitive to salinity.

Steppuhn *et al.* (2001) resulted that under severe salinity (solution electrical conductivity of 24.9 dS m^{-1}); neither the field peas nor the dry bean produced any grain. Some changes in photosynthetic activities of crops grown under saline condition are being occurred. As soil salinity increases due to saline water applications, water consumption of pea decreased. Photosynthetic activity decreases leading to reduced plant growth, leaf area, chlorophyll content and chlorophyll fluorescence when plants are grown under saline conditions. This decrease affects crop performance in different growth stages (Jamil *et al,* 2007). The aim of this present experiment was to study the physiological responses of pea under saline conditions.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Seeds of ten different genotypes (Asgrow, Jumbo, Lincoln, Merveille de Kelvedon, Purser, Rajao Torpe, Rondo, Snajor Kosep Korai, Wando, and a Local variety), with varying salt tolerance potential, were sown in plastic pots filled with fine sand as growth medium. Seven seeds per pot were sown and after 2 weeks of germination, the plants were thinned to five. The experiment was carried out in the green house of the High Agronomic Institute of Chott Mariem, Sousse, Tunisia. One pot was considered as one replicate). Plants were grown in Hoagland solution under non saline conditions for 30 days after germination. Afterwards, salt treatment was initiated. Sodium chloride (NaCl) was dissolved in double distilled water to obtain final concentration of 0 (Control), 25, 50 and 75 mM.

These salinity levels were screened from a range of salinity treatments in a separate preliminary experiment, and three levels i.e, low salinity (25 mM), intermediate salinity (50 mM) and high salinity (75 mM) was created in current investigation. In this way, a clear performance of tested pea genotypes were evaluated under three saline regimes i.e. low, intermediate and high salt stress. To avoid the osmotic shock the desired salinity levels i.e. 25, 50 and 75 mM were created by gradually increasing the salinity level (25 mM) after one day interval until final concentrations (50 and 75 mM) were reached after three days. These salinity levels were maintained throughout the required duration the experiment by

regularly noting the electrical conductivity (EC) and pH of the rooting medium. The increase or decrease in EC and pH was adjusted with the help of buffer or salt solution of desired concentration. Plants were grown for two weeks under salt stressed conditions. Plants were irrigated with half strength Hoagland solution, 200 mL per pot. The plants were usually watered with Hoagland solution after one day interval but sometime this interval was varied according to the moisture of the rooting medium (sand).

Internodal Distance, Number of Leaves, Fresh and Dry Biomass

Internodal distance in each plant was measured with the help of measuring tape in centimeters. Number of leaves were also measured for each plant. Fresh biomass of each plant was taken with the help of electric balance. Average of fresh biomass was calculated for each treatment. Dry biomass of whole plant was measured after keeping it in an oven at 70°C for 72 hours. Dry biomass were taken using digital electric balance and means were calculated for each treatment.

Photosynthetic Activity and Transpiration Rate

The photosynthetic activity (Pn), transpiration rate (E) were determined on intact fully matured leaves (Balal *et al,* 2012). Measurements were performed from 12.00 to 14.00 a.m. with following specifications/adjustments: molar flow of air per unit leaf area 403.3 mM $m^{-2}s^{-1}$, atmospheric pressure 91.9 kPa, water vapor pressure into chamber ranged from 5.0 to 6.9 mbar (PAR) at leaf surface was maximum up to 1913 (mol m^{-2} s^{-1}), temperature of leaf ranged from 29.7 to 35.6°C, ambient temperature ranged from 26.9 to 29.7°C, ambient CO_2 concentration was 451 mol mol^{-1}.

Chlorophyll Contents

Chlorophyll content is measured in 3 leaves for each concentration of Na Cl and for each genotype. It is extracted by homogenizing and boiling 1 g of fresh weight leaves in 35 ml ethanol 96%. After centrifugation (10 min at 4.000 g), the chlorophyll content is determined spectro-photometrically from the ethanolic supernatant at 654 nm, as described by Wintermans *et al.* (1965). Ratio Fv/Fm is measured in 3 leaves for each concentration of hydrogen peroxide or thiourea at the flowering period of potato by using a rotary system type FIM 1500.

Na+, K+ Determination

The digested root samples were analyzed for Na+ and K+ by flame photometer (Jenway PFP-7, UK). A graded series of standards (ranging from 10 to 100 mg L-1) of Na+ and K+ was prepared and standard curves were drawn. The values of Na+ and K+ from flame photometer were compared with standard curve and original quantities were computed.

Statistical Analysis

The experiment was laid out in two factors (salinity and genotypes) factorial arrangement under Completely Randomize Design (CRD). The data was analyzed statistically by using two-way analysis of variance with the statistical software (Statistix 7.1) and comparisons with P-values 0.05 were considered significantly different by using HSD values.

RESULTS

Generally all examined parameters were gradually decreasing with increasing NaCl osmotic potential (Table 1-6) except Na and K content (Tab. 6,7). Growth attributes were negatively affected by NaCl osmotic potentials. The highest and the lowest internodal distance were gained in 0.0 mM (control) 7.3 cm (Merveille de Kelvedon) and 1.3 cm repectively

(Table 1). The highest fresh biomass (12.6 g) (Control) and the lowest one (4.6 g) (75 mM Na Cl) were obtained with genotype Purser. This genotype gave also the highest value of dry Biomass with control (3.19 g) and the lowest value (1.32 g) with 75 mM Na Cl treatment (Table 2). Khan *et al.* (1997), Anantharaju and Muthiah (2007) and Shitole and Dhumal (2012) reported that the internodal distance, fresh and dry biomass were reduced by increasing NaCI concentration.

Table 1: Effect of Na Cl on Internodal Distance (cm)

	Na Cl (mM)			
Genotypes	0	25	50	75
Asgrow	5.8±1.1	4.6±1.5	3.3±0.9	1.8±0.3
Jumbo	6.7±1.5	5.8±1.3	4.7±1.2	2.3±0.5
Lincoln	6.3±1.4	5.2±1.6	4.1±0.8	2.7±0.8
Merveille de Kelvedon	7.1±1.9	6.2±2.7	3.8±0.6	1.9±0.4
Purser	7.3±2.2	6.5±2.6	3.7±0.7	2.4±0.6
Rajai Torpe	5.9±1.3	4.4±1.5	3.2±0.8	1.5±0.3
Rondo	6.4±2.3	5.1±1.9	3.9±0.7	1.3±0.2
Snajor Kosep Koari	5.1±1.7	3.9±0.9	2.8±0.5	1.9±0.9
Wando	6.6±2.5	5.2±1.4	3.7±0.6	2.1±0.8
Local variety	5.7±1.6	4.5±1.6	3.2±0.4	2.5±0.7

Table 2: Effect of Na Cl on Fresh and Dry Biomass (g)

	Fresh Biomass (g)				Dry Biomass (g)			
	Na Cl (mM)				Na Cl (mM)			
Genotypes	0	25	50	75	0	25	50	75
Asgrow	10.8±4.6	9.5±4.7	7.3±2.9	5.6±2.5	2.71±0.54	1.74±0.65	1.18±0.19	1.01±0.25
Jumbo	11.8±5.6	9.1±3.4	6.8±2.3	4.9±2.1	2.63±0.51	1.13±0.53	1.19±0.21	1.23±0.36
Lincoln	11.9±4.5	10.3±3.7	8.1±2.9	6.3±1.9	2.96±0.65	2.55±0.59	2.07±0.18	1.58±0.49
Merveille de Kelvedon	12.1±3.2	10.8±3.9	8.4±2.8	6.5±1.3	2.87±0.48	2.59±0.76	2.18±0.31	1.46±0.35
Purser	12.6±3.7	8.5±4.7	6.8±1.9	4.6±1.5	3.19±0.81	2.48±0.45	1.67±0.46	1.32±0.45
Rajai Torpe	11.4±2.8	9.4±4.3	7.1±2.7	5.6±1.9	2.48±0.96	1.29±0.48	1.87±0.53	1.61±0.37
Rondo	11.7±3.9	9.7±4.5	7.5±2.9	5.8±2.7	2.55±0.94	1.71±0.32	1.36±0.49	1.13±0.24
Snajor Kosep Koari	11.3±3.2	10.8±3.3	8.7±2.2	6.3±2.3	2.24±0.79	1.52±0.29	1.45±0.27	1.11±0.28
Wando	10.9±3.8	9.7±4.1	7.9±1.8	5.8±2.7	2.16±0.64	2.65±0.56	1.27±0.32	1.08±0.21
Local variety	10.4±4.1	10.4±4.5	8.3±2.2	6.1±2.3	2.09±0.58	2.39±0.41	2.19±0.31	2.01±0.53

On the other hand, the highest values of number of leaves (83) and the lowest one (55) were detected in control and 75 mM Na Cl treatments respectively with genotype Purser (Table 3). This genotype gave also maximum photosynthesis (8.9 Pn) and Transpiration (30.1) rates with control tratment, while minimum ones (2.2 Pn and 9.6) were obtaines with Asgrow and Rondo genotypes in 75 mM treatments as shown in Table 4. Sharma (2010 pointed that number of leaves decreased significantly with increased salinity. NaCl negatively affected photosynthesis and transpiration rate.

Table 3: Number of leaves/plant

	Na Cl (mM)			
Genotypes	0	25	50	75
Asgrow	73±13	64±9	59±7	56±11
Jumbo	74±12	64±8	55±8	53±7
Lincoln	81±10	74±11	69±9	60±8
Merveille de Kelvedon	79±9	76±13	68±12	62±11

Table 3: Contd.,				
Purser	83±11	70±9	61±10	55±7
Rajai Torpe	75±10	68±12	62±11	57±12
Rondo	77±13	71±15	65±14	54±7
Snajor Kosep Koari	79±12	72±13	69±15	56±12
Wando	72±10	69±8	67±10	54±14
Local variety	71±9	68±16	65±14	58±16

Table 4: Effect of Na Cl on Photosynthetic rate (Pn) and on Transpiration Rate

	Photosynthesis rate (Pn)				Transpiration rate (Pn)			
Na Cl (mM)	0	25	50	75	0	25	50	75
Asgrow	8.3±2.6	4.5±1.6	3.4±0.9	2.2±0.3	25.2±1.3	18.4±1.9	16.4±1.1	9.7±0.8
Jumbo	8.6±1.7	5.7±1.9	3.9±0.8	2.3±0.2	29.4±4.9	18.7±1.7	15.3±1.8	10.5±0.7
Lincoln	8.2±2.8	6.8±2.3	4.9±0.6	2.7±0.1	27.8±5.3	19.1±2.3	16.8±2.4	13.3±1.4
Merveille de Kelvedon	8.1±2.3	6.7±3.9	4.8±0.4	2.8±0.4	28.6±3.7	20.9±3.8	17.4±1.6	13.6±1.7
Purser	8.9±1.5	5.8±2.6	3.3±0.3	2.6±0.7	30.1±3.8	22.3±1.4	13.2±2.7	10.9±0.8
Rajai Torpe	7.4±2.7	5.2±2.5	2.8±0.2	2.5±0.4	25.8±4.1	19.7±1.5	14.3±2.9	11.1±2.1
Rondo	7.6±2.8	5.1±2.4	3.7±0.6	2.6±0.5	24.3±3.6	18.4±2.2	12.6±2.7	9.6±0.7
Snajor Kosep Koari	7.4±3.4	5.2±1.8	3.6±0.7	2.4±0.4	22.8±2.7	16.8±0.7	13.3±2.6	10.7±0.6
Wando	7.5±2.5	6.7±2.7	4.8±0.9	2.3±0.2	23.7±2.9	19.4±1.2	15.1±1.5	12.1±1.7
Local variety	7.9±3.4	6.6±1.4	4.7±0.8	2.9±0.1	25.6±2.4	20.3±1.6	16.2±1.9	13.2±1.1

According to the results from this study, considerably decrease was observed in chlorophyll a and b, depending on the level of NaCl concentration (Table 5). In fact, minimum total chlorophyll content (1.17 mg/ g of fresh biomass) was signalled with Asgrow genotype in 75 mM Na Cl tratment. Othewise control traetment allow to have maximum total chlorophyll content with Lincoln genotype (3.77 mg/ g fresh biomass) (Table 6). Similar results were reflected by Anantharaju and Muthiah (2007). Also, Jabeen *et al,* (2003) reported decline in total chlorophyll content under saline conditions.

Table 5: Effect of Na Cl on Chlorophyll Content

	Chlorophyll a				Chlorophyll b			
Na Cl (mM)	0	25	50	75	0	25	50	75
Asgrow	2.2±0.068	2.0±0.095	1.8±0.033	0.9±0.059	0 .94±0.032	0.72±0.039	0.58±0.065	0.27±0.03
Jumbo	2.1±0.029	1.8±0.086	1.3±0.043	1.1±0.037	1.14±0.017	0.81±0.065	0.57±0.051	0.28±0.08
Lincoln	2.6±0.087	2.1±0.054	1.8±0.098	1.5±0.047	1.17±0.018	0.97±0.045	0.62±0.071	0.31±0.011
Merveille de Kelvedon	2.5±0.054	2.2±0.035	1.5±0.054	1.3±0.068	1.11±0.034	0.99±0.098	0.71±0.048	0.43±0.061
Purser	2.4±0.038	2.1±0.095	1.7±0.046	1.2±0.023	1.13±0.012	0.81±0.096	0.67±0.074	0.34±0.032
Rajai Torpe	2.3±0.012	1.9±0.098	1.5±0.032	1.1±0.046	0.81±0.042	0.58±0.054	0.38±0.013	0.16±0.056
Rondo	2.4±0.043	2.1±0.084	1.1±0.076	0.9±0.051	1.13±0.025	0.81±0.028	0.49±0.096	0.29±0.087
Snajor Kosep Koari	2.2±0.067	2.0±0.047	1.7±0.021	1.2±0.075	0.93±0.059	0.78±0.082	0.58±0.071	0.23±0.060
Wando	2.5±0.078	2.2±0.038	1.4±0.041	1.2±0.064	0.97±0.021	0.79±0.063	0.53±0.063	0.25±0.019
Local variety	2.4±0.99	2.1±0.065	1.7±0.055	1.3±0.032	1.03±0.032	0.81±0.091	0.51±0.019	0.26±0.036

Table 6: Effect of Na Cl on Total Chlorophyll Content (mg/g Fresh Matter)

	Na Cl (mM)			
Genotypes	0	25	50	75
Asgrow	3.14±0.089	2.72±0.099	2.38±0.051	1.17±0.087
Jumbo	3.24±0.045	2.61±0.092	1.87±0.069	1.38±0.061
Lincoln	3.77±0.091	3.07±0.071	2.42±0.097	1.81±0.064
Merveille de Kelvedon	3.61±0.076	3.19±0.056	2.21±0.071	1.73±0.085
Purser	3.53±0.057	2.91±0.091	2.37±0.065	1.54±0.043
Rajai Torpe	3.21±0.041	2.48±0.097	1.88±0.059	1.26±0.067
Rondo	3.53±0.033	2.91±0.093	1.59±0.091	1.19±0.073
Snajor Kosep Koari	3.13±0.087	2.78±0.069	2.28±0.048	1.43±0.092

Table 6: Contd.,				
Wando	3.47±0.093	2.99±0.052	1.93±0.063	1.45±0.084
Local variety	3.21±0.098	2.91±0.087	1.21±0.078	1.56±0.056

The genotypes Jumbo and Purser showed maximum content of Na in leaf (7.68 and 7.37 respectively) under higher salinity level (75 mM Na Cl) in leaf. However, minimum Na leaf content was obtained in control treatment with Merveille de Kelvedon genotype (1.58). This later gave also the minimum value of root Na content with genotype Jumbo (1.09 and 1.04 respectively) (Table 7). Shitole and Dhumal (2012) reported that increasing NaCl caused increase in Na content. Similar results were reported by Farahbakhsh (2012).

Table 7: Effect of Salinity on Na Contents of Both Leaves and Roots

	Leaf				Root			
Na Cl (mM)	0	25	50	75	0	25	50	75
Asgrow	1.84±0.007	3.12±0.04	4.43±0.06	7.13±2.3	1.11±0.16	3.12±0.12	4.57±0.54	8.03±1.03
Jumbo	1.73±0.012	3.14±0.009	4.58±0.02	7.68±1.7	1.09±0.27	3.58±0.24	6.61±0.67	7.74±1.12
Lincoln	1.61±0.018	3.79±0.05	3.45±0.12	6.26±1.9	1.62±0.19	4.04±0.35	5.29±0.98	7.63±0.94
Merveille de Kelvedon	1.58±0.009	4.19±0.07	5.21±0.05	6.17±1.5	1.04±0.21	2.87±0.17	4.71±0.37	6.91±0.83
Purser	1.59±0.021	2.25±0.007	5.48±0.07	7.37±1.1	1.81±0.32	3.09±0.45	4.59±0.65	6.07±0.87
Rajai Torpe	1.63±0.082	3.19±0.006	5.51±0.11	6.8±0.9	1.91±0.25	3.19±0.39	5.26±0.28	7.16±0.69
Rondo	1.71±0.091	2.09±0.015	4.07±0.14	6.92±0.8	1.36±0.47	4.09±0.17	6.18±0.31	7.58±0.28
Snajor Kosep Koari	1.91±0.054	3.61±0.020	4.19±0.08	6.97±1.3	1.24±0.29	2.29±0.75	5.47±0.37	7.92±0.73
Wando	1.84±0.095	2.97±0.012	5.14±0.12	6.31±0.7	1.04±0.65	2.91±0.54	4.79±0.89	6.87±0.91
Local variety	1.74±0.033	3.49±0.015	4.13±0.16	5.69±0.6	1.74±0.98	4.52±0.46	5.93±021	6.24±0.88

Increasing salinity in irrigation water from 0 to 75 mM NaCl increased both the leaf and root K concentration in all the pea genotypes (Table 8). The lowest reduction in leaf K content was recorded for local variety and Rajai Torpe (11.71 and 12.07 respectively) in control treatment. In roots minimum values was also recodered in control tratment with Rajai Torpe and Purser (12.17 and 11.64 respectively). Salt stress also caused a remarkable increas in leaf and root Na and K contents in all the pea). Similar results were reported by Kaymakanova, 2009 ; Flowers *et al.* 2010 and Cokkizgin, 2012).

Table 8: Effect of Salinity of K Contents of Both Leaves and Roots

	Leaf				Root			
Na Cl (mM)	0	25	50	75	0	25	50	75
Asgrow	12.69±0.83	16.52±0.93	19.64±2.45	23.32±3.56	13.78±1.36	16.11±1.26	18.13±1.78	22.63±3.48
Jumbo	13.41±0.74	15.68±1.12	20.46±2.18	24.16±3.54	14.09±2.87	17.13±1.95	19.56±1.34	23.37±4.87
Lincoln	12.58±0.86	16.81±0.97	18.78±1.45	21.43±3.78	13.41±2.17	16.08±1.88	17.05±1.56	18.76±3.76
Merveille de Kelvedon	13.04±0.79	17.75±1.65	20.25±1.94	24.16±3.96	12.83±1.93	15.37±1.25	18.27±2.78	19.15±2.65
Purser	12.67±0.53	14.63±0.86	19.87±1.66	24.13±2.94	11.64±1.64	14.48±1.65	19.51±1.99	21.51±2.42
Rajai Torpe	12.07±0.87	15.91±0.93	20.16±1.65	25.34±2.74	12.17±1.43	15.61±2.31	18.47±1.74	22.43±2.61
Rondo	14.15±1.05	19.45±0.95	21.35±1.39	24.76±2.33	13.93±1.98	16.33±2.54	19.41±1.54	21.27±1.94
Snajor Kosep Koari	13.28±0.65	17.24±1.05	21.26±1.81	25.24±3.21	12.25±1.11	14.62±1.56	18.73±1.79	23.69±3.78
Wando	12.28±0.68	15.39±1.54	18.34±1.54	22.78±3.89	11.72±2.34	13.91±1.74	17.07±1.56	20.18±2.23
Local variety	11.71±0.93	14.31±0.73	17.28±1.93	20.47±2.98	10.97±1.91	15.61±1.28	17.24±1.43	18.31±2.78

DISCUSSIONS

Salt stress significantly affected the various growth attributes such as internodal distance, fresh and dry biomass, number of leaves/plant plant biomass. It also affected photosynthesis, transpiration and accumulation of Na^+ and K^+ on both roots and leaves. On the basis of these important indicators of salt stress, differences in salt tolerant and salt sensitive genotypes can easily be investigated. High internodal distance, fresh and dry biomass, number of leaves/plant, high chlorophyll content of genotypes Lincoln, Merveille de Kevedon, Wando and local variety in present study, contribute in thier potential to resist the salt stress, so indirectly play a vital role for better growth and productivity (Carpici *et al,* 2009). Therefore, poor internodal distance, fresh and dry biomass, number of leaves/plant, high chlorophyll content of genotypes Asgrow and Jumbo might have been due to high accumulation of toxic salts i.e. Na^+ and Cl^-, which may lowered their water absorption potential. Various reports indicate that salt tolerance is development stage specific processes because one developmental stage may be drastically affected while other exhibit tolerance to salts (Johnson *et al,* 1992). The results regarding internodal distance, fresh and dry biomass, number of leaves/plant, high chlorophyll content has strongly confirmed the findings of Ahmad and Khan (2010). In current investigation, salt stress caused a marked reduction in plant fresh and dry weights. This reduction in plant biomass might have been due to decreases in water potential of growth medium due to high salinity, which leads to reduction in cell turgor. This low cell turgor inhibited the cell elongation, cell division and internodal distances so resultantly less formation new dry biomass. The best performance of Lincoln, Merveille de Kelvedon and local variety may be due to their well cell turgor maintenance in response to salt stress while Asgrow and Jumbo could not maintained suitable cell turgor potential so exhibited plants with low fresh and dry weights. The results regarding the plant biomass confirmed the findings of Ashraf *et al,* (2008). On the other hand, the better growth of Lincoln, Merveille de Kelvedon and local variety in terms of high intermodal distance and plant fresh and dry biomass may also be linked with high cell differentiation and cell elongation under salt stress.

In present investigation salt stress significantly reduced the photosynthetic activity (Pn) in both tolerant and sensitive pea genotypes but maximum drastic effect of salinity was observed in non tolerant Asgrow and Jumbo. This decrease in Pn may be attributed to destroy of chlorophyllous and mesophyllous membranes, which ultimately resulted in reduced Pn. Therefore, reduced Pn may also be the solid reason of reduced biomass under saline conditions. Since the genotypes, Lincoln, Merveille de Kelvedon and local variety exhibited the high Pn rates as compared to Asgrow and Jumbo under salinity so they (climax Lincoln, Merveille de Kelvedon and local variety) also had high biomass production. As salinity influence various Pn related processes i.e. availability of CO_2 (Flexas *et al,* 2007), disturbances in photosynthetic metabolism (Lawlor and Cornic, 2002) and irregularities in the photochemical system apparatus (Souza *et al,* 2004) so it ultimately leads to reduction in Pn. Hura *et al,* (2007) also reported that salt stress cause redution in Pn. Literature also depicted that salt stress effects the stomatal functioning by limiting the availability of K+ ions to leaf. These K+ ions have vital role in stomatal opening and closing so salts stress disturb the activity of photosynthetic apparatus by causing the disturbances in gaseaous exchange. Therefore, this factor can not be ignored in case of highly reduced Pn activity of Asgrow and Lincoln. High Pn of tolerant genotypes (Lincoln, Merveille de Kelvedon and local variety) could have been closely associated with the maximum availability of K+ in leaf tissue, which facilitated the efficient gaseous exchange and resulted in high Pn.

Whereas, sensitive pea genotypes (Asgrow and Jumbo) accumulated high Na+ and low ratios of K+ in their leaves, resultantly functioning of stomata become disturbed which ultimately caused the improper gaseous exchange and

reduced photosynthetic activity. Similarly, under saline regimes, irregular functioning of stomata and less availability of intercellular CO_2 enhanced the photochemical damages due to high light energy at PSII under low CO_2 assimilation rates. In this study photosynthetic activity may also be related to the disturbances in photochemical reactions due to excessive ion toxicity and this statement is confirmed by the findings of Tezara *et al,* (2005). From the above facts, it is obvious that high ion toxicity, high osmotic stress, disintegration of membranes, disturbances in stomatal functioning and less availability of K^+ is the key factors that may be the reason of reduced photosynthetic activity in tested pea genotypes.

Osmotic stress is the significant symptom of salt stress. The genotypes having good osmotic adjustment potential showed less drastic effects of salinity. The plants adopt different mechanisms for osmotic adjustment under saline environments and among these production of compatible solutes is more important. Among various compatible solutes, proline has vital position and occurs in higher plants in more ratios than amino acids. It promotes the deposition of useable nitrogen and enhances the membrane stability under salt stress. Similar kind of findings had been reported by Hassine and Lutts (2010). Salinity also has significant influence on the nutritional status of the plant therefore nutrient regulation is a vital process, which is closely linked with the salt tolerance potential. It is well documented that salt stress elevates the sodium (Na^+) in plant parts while suppresses the concentration of potassium (K^+) (Akram $^{et\ al,}$ 2010). In the present investigation, all the tested pea genotypes exhibited an increase in the leaf Na^+ and K+ contents. Since, Lincoln, Merveille de Kelvedon and local variety showed the minimum concentration of Na+ and K+ in their leaves while on the other hand, genotypes such as Asgrow and Jumbo, showed highest leaf Na^+ and lowest leaf K^+ contents. Therefore, it is concluded that there is a positive correlation between leaf Na^+ and K^+, and salt tolerance is highly associated with ratios of these ions. This difference in Na^+ and K^+ of pea genotypes may be due to their genetic variability and root permeability for these ions. The salt tolerant plants might have been transported less amounts of toxic ions like Na^+ to the upper parts (leaf and shoot) because they stored maximum amounts of these ions in their roots. It is an adaptation to withstand saline conditions while salt sensitive plants do not have such kind of mechanism. Similar kinds of observations were noted by Balal (2010) in salinized citrus rootstocks. So, it may also be the reason of reduced leaf Na in Lincoln, Merveille de Kelvedon and local variety. This observation is in agreement with the findings of Khayat *et al,* (2010). Overall, it can be concluded that high Pn, low ratios of toxic ions and high osmotic adjustment is the major difference between salt tolerant and non tolerant pea genotypes.

Detailed analysis of xylem sap composition in relation to salt stress can provide information about the structure and complexity of the physiological mechanisms that are correlated with specific ionic and biophysical stresses in the rhizosphere, and reveal unexpected or previously uncharacterized biochemical interactions. Xylem sap profiling is likely to provide physiological markers to assist root-targeted breeding for resistance to stress salt. Salinity as it's established later affect plant productivity by reducing the photosynthetic area by inhibiting cell division and cell expansion rates during leaf growth and by affecting developmental programs regulating leaf emergence, the production of lateral primordia, and the formation of reproductive organs (Munns, 2002). Whether water status, hormonal regulation, or supply of photosynthate exerts dominant control over growth of plants in saline soil is an issue that has been hotly debated. Over the time scale of days, there is much evidence to suggest that hormonal signals rather than water relations are controlling growth in saline soils (Munns, 2011; Pe´rez-Alfocea *et al,* 2010) because leaf expansion in saline soil only transiently (< 24 h) responds to increased leaf water status (Munns, 2002). Therefore, rootderived chemical signals limit leaf growth in saline soil.

Because salt stress can increase xylem ABA concentrations by several orders of magnitude, there has also been interest in whether ABA conjugates or metabolites can affect shoot physiology. Although phaseic acid (the metabolic product of 8-OH hydroxylation of ABA) can also be detected in xylem sap if leaf water deficits occur, it exerts a negligible antitranspirant activity. ABA glucose ester (ABA-GE) occurs in the xylem sap of several plants (Sauter *et al,* 2002) and the intercellular washing fluid of barley primary leaves contains a b-glucosidase activity (Dietz *et al,* 2000) that releases ABA from ABA-GE in the leaf apoplast. The activity of these b-glucosidases increased with salt stress (Dietz *et al,* 2000) so that xylem-transported ABA-GE is probably a source of stress-induced apoplastic ABA in the leaf. Apoplastic alkalinization, in response to local atmospheric conditions or soil drying-induced changes in xylem sap pH, will result in the release of chloroplastic ABA to the apoplast (Wilkinson and Davies, 2008) and can initiate stomatal closure prior to any increase in xylem ABA concentration.

In response to soil flooding, plants increased xylem ACC Aminocyclopropane Carboxylic Acid (ACC) (the immediate precursor of ethylene) delivery prior to the classical physiological and morphological responses of petiole ethylene production and epinastic curvature. Supplying xylem ACC concentrations typically found in xylem sap of flooded tomato plants (at least 3 lM) to shoots detached from welldrained plants induced petiole ethylene evolution and epinasty (Bradford and Yang, 1980), suggesting that rootsourced ACC was responsible for shoot responses. However, the role of root-sourced ACC as a signal of saline soil is more equivocal.

Salt shock (200 mM NaCl) increased xylem ACC concentrations in hybrid citrange (Citrus sinensis · Poncirus trifoliata) (Gomez-Cadenas *et al,* 1998). Similarly, tomato plants required salinization (at 100 mM NaCl) for 15 days before xylem ACC concentration increased (Albacete *et al,* 2008), with further work demonstrating that the rootstock could double the xylem ACC concentrations detected (Albacete *et al,* 2009). Because ACC is likely phloemmobile (because it is a weak acid similar to ABA) and recirculates from leaves to roots, further work is required to determine whether these differences are due to intrinsic differences in root ACC synthesis, or due to differences in shoot ionic relations mediating ACC transport to the roots of pea. Salt stress induction decreased leaf water potential to - 3.0 MPa, increased xylem ACC concentration. A more moderate soil salt treatment of pea (Pisum sativum) which imposed a relatively stable soil water status (that did not exceed a matric potential of - 0.25 MPa) also roughly doubled xylem ACC concentration (Belimov *et al,* 2009). Nevertheless, additional work is necessary to establish whether increased xylem ACC concentration is a typical response to salt stresss, especially because the prevailing view in the literature that salt soil does not enhance leaf ethylene evolution.

On the other hand, it has been established that under salt stress, both leaf growth and delayed senescence were positively correlated with rootstock-mediated zeatin (Z) and K^+ concentrations in the leaf xylem sap and also with hormonal ratios between CKs and ACC (Z/ACC and Z + ZR/ACC), whereas the ratio ACC/ABA was negatively correlated with leaf biomass (Ghanem *et al,* 2008). It was suggested that early hormonal signals coming from the roots positively (CKs, ABA) or negatively (ACC) influenced leaf growth, while lower xylem CK concentrations or higher ACC concentrations promoted leaf senescence (Pe´rez-Alfocea *et al,* 2010), and the balance of different compounds may be crucial in regulating physiological responses. Maintaining growth while minimizing senescence may provide more energy to maintain ionic homeostasis by acting on both root ion uptake (K^+) and efflux (Na^+) and by diluting toxic ions through growth (Pe´rez-Alfocea *et al,* 2010). Although the bioactive cytokinins (tZ, tZR, IP, IPR) seem key in regulating growth and senescence processes as described above, the role of other CK groups that significantly increase under stress

conditions such as cis-CKs should be investigated (Ghanem *et al,* 2011).

Changes in xylem ion concentration in response to salinity have almost exclusively focused on sodium and chloride ion concentrations. Nevertheless, a recent study indicates that a 7-day exposure of Brassica oleracea to 80 mM NaCl (Fernandez- Garcia *et al,* 2011) increased xylem sap potassium concentration (1.3-fold), calcium concentration (1.6-fold), and nitrate concentration (3.7-fold). Increases in potassium concentrations were also reported in the xylem sap of salinized tomato plants, while Ca^{2+} and Mg^{2+} were not clearly affected after 3 weeks under 100 mM NaCl stress (Pe´rez-Alfocea *et al,* 2000). The causes (changes in nutrient uptake, xylem loading, or sap flow rate) and physiological significance of these changes requires further work. However, because salinity usually decreases transpiration rates (Munns, 2011), these changes in concentration may not actually increase delivery rates of these ions to the shoot, consistent with many observations of decreased tissue nutrient status in salinized plants (Hu *et al,* 2005). Furthermore, it should be noted that xylem sodium and chloride ion concentrations were increased by at least an order of magnitude more in tomato and oilseed rape (Fernandez-Garcia *et al,* 2011).

REFERENCES

1. Ahmed MZ and MA Khan, 2010. Tolerance and recovery responses of playa halophytes to light, salinity and temperature stresses during seed germination. Flora, 205: 764-771.
2. Akram M, MY Ashraf, R Ahmad, EA Waraich, J Iqbal and M Mohsan, 2010. Screening for salt tolerance in maize (*Zea mays* l.) hybrids at an early seedling stage. Pakistan Journal of Botany, 42: 141-154.
3. Albacete, A, Martinez-Andujar, C, Ghanem, M. E, Acosta, M, Sanchez-Bravo, J, Asins, M. J, et al. (2009). Rootstockmediated changes in xylem ionic and hormonal status are correlated with delayed leaf senescence and increased leaf area and crop productivity in salinised tomato. Plant Cell Environ 32, 928–938.
4. Anantharaju, P. and Muthiah, A. R. 2007. Effect Of Nacl Salinity Stress On Seed Germination And Seedling Growth Of Chick Pea (*Cicer arietinum* L.). *Legume Res,* 30 (2) : pp. 141-144.
5. Ashraf M, HR. Athar, PJC Haris and TR Kwon, 2008. Some prospective strategies for improving crop salt tolerance. Advances in Agronomy, 97: 45-110.
6. Balal RM, 2010. Morpho-physiological and biochemical characterization and *in vitro* propagation of salt-tolerant citrus rootstocks. Discussion Ph.D thesis. University of Agriculture Faisalabad, Pakistan.
7. Balal RM, Khan MM, Shahid MA, Mattson NS, Abbas T, Ashfaq M, Garcia-Sanchez F, Ghazanfer U, Gimeno V, Iqbal Z (2012) Comparative studies on the physiobiochemical, enzymatic, and ionic modifications in salt tolerant and salt sensitive Citrus rootstocks under NaCl stress. J Am Soc Hor Sci 137:1-10
8. Belimov, A. A, Dodd, I. C, Hontzeas, N, Theobald, J. C, Safronova, V, and Davies, W. J. (2009). Rhizosphere bacteria containing ACC deaminase increase yield of plants grown in drying soil via both local and systemic hormone signalling. New Phytol 181, 413–423.
9. Bradford, K. J, and Yang, S. F. (1980). Xylem transport of 1-aminocyclopropane-1-carboxylic acid, an ethylene precursor, in waterlogged tomato plants. Plant Physiol 65, 322–326.

10. Carpici EB, N Celik and G Bayram, 2009. Effects of salt stress on germination of some maize (*Zea mays* L.) cultivars. African Journal of Biotechnology, 8: 4918-4922.

11. Cerda, M, M. Caro and F. G. Fernandez, 1982. Salt tolerance of two pea cultivars. *Agron. J,* 74: 796–798.

12. Cokkizgin, A. (2012). Salinity Stress in Common Bean (*Phaseolus vulgaris* L.) Seed Germination. Notulae Botanicae Horti Agrobotanici, 40(1): pp. 177-182.

13. Dietz, K. J, Wichert, K, Sauter, A, Messdaghi, D, and Hartung, W. (2000). Characterisation of an extracellular B-glucosidase in barley involved in the hydrolysis of ABA glucose conjugate in leaves. J Exp Botany 51, 937–944.

14. Farahbakhsh, H. (2012). Germination and seedling growth in un-primed and primed seeds of Fenel as affected by reduced water potential induced by NaCl. International Research Journal of Applied and Basic Sciences. Vol, 3 (4), pp. 737-744.

15. Fernandez-Garcia, N, Hernandez, M, Casado-Vela, J, Bru, R, Elortza, F, Hedden, P, et al. (2011). Changes to the proteome and targeted metabolites of xylem sap in Brassica oleracea in response to salt stress. Plant Cell Environ 34, 821–834.

16. Flowers TJ, 2004. Improving crop salt tolerance. Journal of Experimental Botany, 55: 307-319.

17. Flowers, T. J, Gaur, P. M, Gowda, C. L. L, Krishnamurthy, L, Samineni, S, Siddique, K. H. M, Turner, N. C, Vadez, V, Varshney, R. K. and Colmer, T. D, (2010). Salt sensitivity in chickpea. Plant, Cell and Environment, 33: pp. 490-509.

18. Garcia-Sanchez F, J Jifon, MCarvajal and JP Syvertsen, 2002. Gas exchange, chlorophyll and nutrient contents in relation to Na+ and Claccumulation in 'Sunburst' mandarin grafted on different rootstocks. Plant Science, 162:705-712.

19. Ghanem, M. E, Albacete, A, Martinez-Andujar, C, Acosta, M, Romero-Aranda, R, Dodd, I. C, et al. (2008). Hormonal changes during salinity-induced leaf senescence in tomato (Solanum lycopersicum L.). J Exp Botany 59, 3039–3050.

20. Ghanem, M. E, Albacete, A, Smigocki, A. C, Frebort, I, Pospı´s˘ilova, H, Martı´nez-Andu´jar, C, et al. (2011). Rootsynthesised cytokinins improve shoot growth and fruit yield in salinised tomato (Solanum lycopersicum L.). J Exp Botany 62, 125–140.

21. Gomez-Cadenas, A, Tadeo, F,R, Primo-Millo, E, and Talon, M. (1998). Involvement of abscisic acid and ethylene in the responses of citrus seedlings to salt shock. Physiol Plant 103,475–484.

22. Hassine AB and S Lutts, 2010. Differential responses of saltbush *Atriplexhalimus* L. exposed to salinity and water stress in relation to senescing hormones abscisic acid and ethylene. Journal of Plant Physiology, 167: 1448-1456.

23. Hura T, K Hura, M Grzesiak and ARzepka, 2007. Effect of long term drought stress on leaf gas exchange and fluorescence parameters in C3 and C4 pIants. ActaPhysioIogiaePlantarum, 29: 103- 113.

24. Hu, Y. C, Fricke, W, and Schmidhalter, U. (2005). Salinity and the growth of non-halophytic grass leaves: the role of mineral nutrient distribution. Funct Plant Biol 32, 973–985.

25. Jabeen, M, M. Ibrar, F. Azim, F. Hussain and I. Ilahi. 2003. The effect of sodium chloride salinity on germination and productivity of Mung bean (*Vigna mungo* Linn.) *J. Sci. & Tech. Univ. Peshawar,* 27: pp. 1-5.

26. Jamil, M, S. Rehman, J. L. Kui, J. M. Kim, H. S. Kim, E. S. Rha, 2007. Salinity Reduced Growth Ps2 Photochemistry and Chlorophyll Content in Radish. *Sci. Agric. (Piracicaba, Braz.),* 64 (2): 111- 118.

27. Johnson DW, SE Smith and AK Dobrenz, 1992. Genetics and phenotypic relationships in response to NaCl at different developmental stages in alfalfa. Theoretical and Applied Genetics, 83: 833-838.

28. Kaymakanova, M. (2009). Effect of salinity on germination and seed physiology in bean (*Phaseolus vulgaris* L.), 326-329 p. XI Anniversary Scientific Conference, Sofia, Bulgaria.

29. Khan, M. S. A, Hamid. A, Salahuddin, A. B. M, Quasem, A. and M. A. Karim (1997). Effect of sodium chloride on growth, photosynthesis and mineral ion accumulations of different type of rice (*Oryza sativa* L.). J. Agron. Crop Sci, 179: pp. 149-161.

30. Khayat PN, S Jamaati-e-Somarin, R Zabihi-e- Mahmoodabad, AYari, MKhayatnezhad and R Gholamin, 2010. Screening of salt tolerance canola cultivars (*Brassica napus* L.) using physiological markers. World Applied Sciences Journal, 10: 817-820.

31. Lawlor DW and G Cornic, 2002. Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. Plant Cell and Environment, 25: 275-294.

32. Levy Y and JPSyvertsen, 2004. Irrigation water quality and salinity effects in citrus trees. Horticultural Reviews, 30:37-82.

33. Munns, R. (2002). Salinity, growth and phytohormones. In Salinity: environment—plants—molecules. A. La¨ uchli, and U. Lu¨ ttge, eds. (Kluwer Academic Publishers, New York), pp. 271–290.

34. Munns, R. (2011). Plant adaptations to salt and water stress: differences and commonalities. Adv Botanical Res 57, 1–32.

35. Ozdemir, S, 2006. Yemeklik Tane Baklagiller. Hasad YayincilikLtd Sti, Istanbul (Tr).

36. Pe´rez-Alfocea, F, Balibrea, M. E, Alarco´ n, J. J, and Boları´n, M. C. (2000). Composition of phloem and xylem exudates in relation to the salt-tolerance of domestic and wild tomato species. J Plant Physiol 156, 367–374.

37. Pe´rez-Alfocea, F, Albacete, A, Ghanem, M. E, and Dodd, I. C. (2010). Hormonal regulation of source-sink relations to maintain crop productivity under salinity: a case study of root-toshoot signalling in tomato. Funct Plant Biol 37, 592–603.

38. Rachmilevitch S, AB Cousins and AJ Bloom, 2004. Nitrate assimilation in plants shoots depends on photorespiration. Proceedings of the Natural Academic Sciences, 101: 11506-11510.

39. Ramanjulu S and C Sudhakar, 2001. Alleviation of NaCl salinity stress by calcium is partly related to the increased proline accumulation in mulberry (Morusalba L.) callus. Journal of Plant Biology, 28: 203-206.

40. Sauter, A, Dietz, K.-J, and Hartung, W. (2002). A possible stress physiological role of abscisic acid conjugates in

root-to-shoot signalling. Plant Cell Environ 25, 223–228.

41. Shitole, S. M. and Dhumal, K. N. (2012). Effect Of Water Stress By Polyethylene Glycol 6000 And Sodium Chloride On Seed Germination And Seedling Growth Of *Cassia angustifolia*. International Journal of Pharmaceutical Science and Research, Vol. 3(2): pp. 528-531.
42. Souza RP, EC Machado, JAB Silva, AMMA Lagoa and JAG Silveira, 2004. Photosynthetic gas exchange, chlorophyll fluorescence and some associated metabolic changes in cowpea (*Vignaunguiculata*) during water stress and recovery. Environmental and Experimental Botany, 51: 45-56.
43. Steppuhn, H, K. M. Volkmar and P. R. Miller, 2001. Comparing Canola, Field Pea, Dry Bean, and Durum Wheat Crops Grown in Saline Media. *Crop Sci,* 41: 1827–1833.
44. Tezara W, O Marin, E Rengifo, D Martínez and A Herrera, 2005. Photosynthesis and photoinhibition in two xerophytic shrubs during drought. *Photosynthetica*43: 37–45
45. Vural, H, D. Esiyok and I. Duman, 2000. Kultur Sebzeleri. *Ege Universitesi Ziraat Fakultesi Yayini,* Izmir (Tr). Wilkinson, S, and Davies, W. J. (2008). Manipulation of the apoplastic pH of intact plants mimics stomatal and growth responses to water availability and microclimatic variation. J Exp Botany 59, 619–631.
46. Wintermans J. and De Mots A. 1965. Spectrophotometric characteristics of chlorophylls a and b and their photosynthesis in ethanol. *Biochem. Biophys. Acta*, **109**, 448-453.
47. Yang WJ, PJ Rich, JD Axtell, KV Wood, CC Bonhm, G Ejeta, MV Mickelbart and D Rhodes, 2003. Genotypic variation for glycinebetaine in sorghum. Crop Sciences, 43: 162-169.